equal concentrations. NLPs were mixed with a 200-fold molar excess of DHPC/DMPC bicelles (equimolar 6- and 14-carbon acyl chains) in a stopped-flow fluorometer. The rate of lipid transfer was monitored by the appearance of unquenched NBD fluorescence at 520 nm. The observed pseudo-first-order rate constant was surprisingly small (0.26/sec). NLPs did not react with DHPC alone below its critical micelle concentration (cmc). Above the cmc, the reaction was complete within the instrument dead time. Thus, the rate-limiting step is not the reaction of NLPs with DHPC monomers or micelles. Added MSP1E3D1 had no effect on the rate, ruling out free apolipoprotein involvement. The NLP-bicelle mixing rate showed a strong temperature dependence (activation energy = 28 kcal/mol). Near or below the DMPC phase transition temperature, the kinetics were biphasic. The results suggest NLP-bicelle mixing kinetics may be mechanistically similar to lipid mixing via fusion pores.

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Drunken Membranes: How does Ethanol Impact Fusion of Vesicles to Planar Lipid Bilayers?

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Previously we have shown that fusion of vesicles to a membrane is changed by lipid phase as altered by cholesterol and temperature (Lee et al. 2013, Chem Phys Lipids 166:45-54). Ethanol is also known to alter membrane phase behavior.
To investigate the effect of ethanol on fusion rate, we used the Nystatin/Ergosterol (NYS/ERG) fusion assay. We measured fusions per minute using planar membranes containing PE/PC/CHL and vesicles containing PE/PC/PS/ERG. For our initial experiments we used high concentrations of ethanol added to either side of the bilayer. At 1% ethanol an increase was observed, and at 4% fusion rates increased 3 fold compared to control when added to the cis side of the bilayer (same side as vesicles). However, no significant increase was observed when added to the trans side. Similar results were observed with methanol. It also appears that the 2-3 fold increase in fusion rate occurs regardless of whether ethanol is added before or after vesicles.
We hypothesize that this effect of ethanol is to alter lipid phase behavior of the vesicle membrane. To verify the effects of ethanol on membranes we utilized differential scanning calorimetry (nDSC). Solutions of KCl buffer and DPPC vesicles were analyzed with and without ethanol. The DPPC vesicles with ethanol were shown to have a transition state ~0.5°C lower than without ethanol. This is consistent with our hypothesis that adding ethanol alters lipid fluidity and/or phase behavior.

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Role of Electrostatic Interactions in the Anchoring of Dengue E Protein to Lipid Membranes

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Infection of host cells by Dengue virus occurs in the late endosome, where the viral envelope protein, E, mediates the fusion of the viral and endosomal membranes. The low pH triggers a conformational change that first causes the tip of E to project out from the surface of the virus and bind the endosomal membrane. E forms trimers during this process and further conformational changes in the protein force the viral and endosomal membranes into contact. Binding of E to the endosomal membrane has been largely attributed to the hydrophobic fusion loop at the tip of E, as several residues in this region are highly conserved. However, fusion requires anionic lipids in the endosomal membrane, suggesting that binding of E may also be modulated by electrostatic interactions. Here, we use atomistic molecular dynamics simulations to study the interaction between Dengue E trimer and various lipid membranes composed of POPE, POPC, POPG (anionic), and cholesterol. We use a truncated E trimer consisting of roughly 1/3 of the residues (those near the tip). Truncated E retains the structural features of the full protein and significantly reduces the computational costs. We find that E inserts into all membranes tested regardless of the presence of anionic lipids or cholesterol. During insertion, the dominant interactions occur hydrogen bonds between the positively charged lysines and oxygens of the lipid headgroups. These interactions induce tilting of the protein that is accompanied by a large deformation of the membrane. This local deformation of the membrane (inducing negative curvature) produced by electrostatic interactions may play an important role during fusion. The simulations may also suggest that the hydrophobic residues in the fusion loop make only a small contribution to the binding energy.

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Calcium Sensitive Ring-Like Oligomers of Synaptotagmin: Implications for Regulation of Neurotransmitter Release

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The synaptic vesicle protein synaptotagmin-1 (SYT) is required to couple calcium influx to the membrane fusion machinery. However, the structural mechanism underlying this process is unclear. Using electron microscopy we find an unexpected circular arrangement (ring) of SYT's cytosolic domain (C2AB) formed on lipid monolayers in the absence of free calcium. Rings vary in diameter from 18 nm to 43 nm, corresponding to 11 to 26 molecules of SYT. Continuous stacking of the SYT rings occasionally converts both lipid monolayers and liposomal bilayers into protein-coated tubes. Helical reconstruction of the SYT tubes shows that the C2B interacts with the membrane and is involved in ring formation, while the C2A domain points radially outwards. SYT rings are rapidly disrupted by physiological concentrations of free calcium but not by magnesium. Assuming that calcium-free SYT rings are physiologically relevant, these results suggest a simple and novel mechanism by which SYT regulates neurotransmitter release: the ring acts as a spacer to prevent the completion of SNARE complex assembly thereby clamping fusion (in the absence of calcium). When the ring disassembles in the presence of calcium, then fusion proceeds unimpeded.

2045-Pos Board B182

Viral Membrane Fusion at Single Pore Resolution

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Fusion between membrane-enveloped viruses and their hosts is a critical step in infection, enabling the virus to release its genome and subsequently hijack the host cell's replication machinery. The genomes of enveloped viruses are packaged into multiple bulky ribonucleoprotein (RNP) particles whose release requires the dilation of the fusion pore - the initial, narrow, connection between fusing membranes. Electrophysiological studies of influenza A hemagglutinin (HA)-mediated fusion between HA-expressing fibroblasts and red blood cells and between HA-fibroblasts and voltage-clamped suspended bilayers comprise the most complete information to date about the dynamics of viral fusion pores. Small pores (~2-5nm) were found to flicker repetitively and/or fluctuate in size prior to terminal dilation. A quantitative understanding of the biophysical driving forces of viral membrane fusion, including membrane contact area, membrane tension and protein fusogen cooperativity, however, remains elusive, and a number of questions - regarding the physical basis of pore flickering and the number of HA trimers required for successful pore dilation - remain open. Here we describe a novel electrophysiological approach, which has been successfully applied to study SNARE-mediated fusion pores, to probe the HA-mediated fusion pore. We voltage-clamped HA-expressing fibroblasts in the cell-attached configuration and included nanodiscs (NDs) in the patch pipette as fusion partners to probe fusion pore nucleation and evolution at single pore resolution. NDs are flat, bilayer discs whose composition and size can be varied. Current through voltage-clamped fusion pores reports pore size with sub-millisecond time resolution. Preliminary data shows that isolated fusion pores flicker, are long-lived (~10s) and are of diameter ~1.5nm.

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Effect of Cholesterol Depletion on HA Distribution in the Viral Membrane of Influenza

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Influenza virus enters cells via a process of membrane fusion mediated by the hemagglutinin coat protein. We have previously shown that depletion of cholesterol from the viral envelope increases the rate of fusion despite decreasing fusion efficiency. Furthermore, this increased rate of fusion is only observed when cholesterol is removed from the viral envelope, not in the target membrane. Here we use cryo-electron microscopy to measure how the lateral distribution of hemagglutinin in influenza virions changes upon cholesterol extraction. Following removal of cholesterol from the viral envelope, hemagglutinin trimers move closer together. We estimate that HA trimer-trimer spacing decreases from 92.6 angstroms to 84.9 angstroms. Upon re-addition of cholesterol, hemagglutinin trimer spacing expands once